ISOLEUCYL-ALANYL-*p*-AMINOBENZOIC ACID ETHYL ESTER. A COMPOUND SHOWING THE ACTIVITY OF JUVENILE HORMONE AND THE PROPERTIES OF A LOCAL ANESTHETIC

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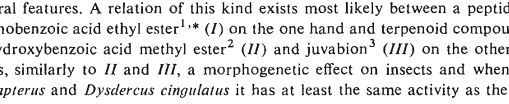
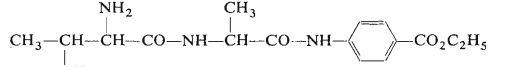
Biological effects of a similar type share rather often compounds with structures resembling only in general features. A relation of this kind exists most likely between a peptide, isoleucyl-alanyl-*p*-aminobenzoic acid ethyl ester[1,*] (*I*) on the one hand and terpenoid compounds, geranyl ether of *p*-hydroxybenzoic acid methyl ester[2] (*II*) and juvabion[3] (*III*) on the other. Tripeptide ester *I* shows, similarly to *II* and *III*, a morphogenetic effect on insects and when tested with *Pyrrhocoris apterus* and *Dysdercus cingulatus* it has at least the same activity as the well known



I



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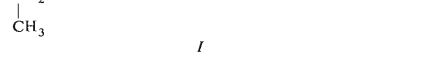

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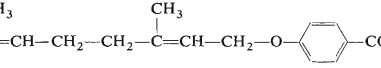

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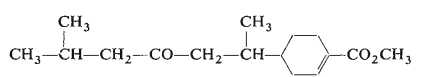

V

* All proteinogenic amino acids are of L-configuration.

juvabion. It also resembles juvabion in the specificity of the effect. Tripeptide ester *I*, however, resembles in structure, among others, also the local anesthetics of the basic anilide type, such as *e.g.* "xylonest" *IV* and "hostacaine" *V*. It was therefore considered interesting to find out whether *I* is active also in this respect. The possibility of obtaining a positive result was considered somewhat skeptically since ester *I* contains a primary amino group(cf^{4}) in its hydrophilic moiety. Orienting pharmacologic studies* showed, however, that tripeptide ester *I* possessed a considerable activity. In the infiltration anesthesia it was approximately as active as "novocaine" and was also active when subjected to the cornea test, eventhough less than cocaine. Ester *I* shows thus not only a hormonal effect (juvenile hormone effect) but also an effect of a local anesthetic. At this instance, however, no conclusions can be made on the possible causal relation between these two properties.

The synthesis of isoleucyl-alanyl-*p*-aminobenzoic acid ethyl ester, which had been mentioned briefly in the preceding report[1], was effected by stepwise synthesis from the carboxyl terminus, using dicyclohexylcarbodiimide as condensing agent and a solution of hydrogen bromide in glacial acetic acid to remove the protecting (benzyloxycarbonyl) groups.

EXPERIMENTAL

The melting points were determined on a Kofler block and were not corrected. The optical activity was measured in an objective polarimeter.

Benzyloxycarbonylalanyl-*p*-aminobenzoic Acid Ethyl Ester

Benzyloxycarbonylalanine (2·23 g, 0·01 mol) and *p*-aminobenzoic acid ethyl ester (1·65 g, 0·01 mol) were dissolved in 25 ml of ethyl acetate. Dicyclohexylcarbodiimide (2·06 g, 0·01 mol) in 5 ml of ethylacetate was added to the solution at 0°C. The reaction mixture was set aside for 1 h at 0°C and for 14 h at room temperature. Thereafter, it was heated to boil for a short while, then cooled down to room temperature, and dicyclohexylurea which had separated was filtered off by suction. The filtrates were taken to dryness at reduced pressure and the residue was recrystallized from a mixture of ethyl acetate and light petroleum. Yield 3·1 g (84%), m.p. 158—159°C, $[\alpha]_D^{25}$ −47·3° (*c* 2, methanol). For $C_{20}H_{22}N_2O_5$ (370·4) calculated: 64·85% C, 5·99% H, 7·56% N; found: 64·91% C, 5·88% H, 7·44% N. Recorded data[5]: m.p. 155°C and 152—153°C, $[\alpha]_D^{23}$ −43·1° and −42·2° (*c* 1, ethanol) for a product prepared by carbobenzoxylation of alanyl-*p*-aminobenzoic acid ethyl ester and for a product prepared by dicyclohexylcarbodiimide synthesis, respectively.

Alanyl-*p*-aminobenzoic Acid Ethyl Ester Hydrobromide

Benzyloxycarbonylalanyl-*p*-aminobenzoic acid ethyl ester (3·2 g, 8·65 mmol) was suspended in 10 ml of a solution (approximately 35%) of hydrogen bromide in glacial acetic acid. After the ester had dissolved (approximately 5 min) the hydrobromide of alanyl-*p*-aminobenzoic acid ethyl ester was precipitated with ether. The hydrobromide was filtered off by suction and dried. Yield 2·56 g (93%). This product was used directly for subsequent synthesis. For analysis, the hydrobromide was recrystallized from ethanol–ether. M.p. 252—254°C (slight sintering from 245°C), $[\alpha]_D^{23}$ +25·2° (*c* 2·6, water). For $C_{12}H_{16}N_2O_3$.HBr (317·2) calculated: 45·56% C, 5·40% H, 8·83% N; found: 45·48% C, 5·35% H, 8·70 % N.

* The activity determinations were performed by MUDr. F. Hradil, Research Institute for Pharmacy and Biochemistry, branch Rosice/Labem.

Benzyloxycarbonylisoleucyl-alanyl-*p*-aminobenzoic Acid Ethyl Ester

Alanyl-*p*-aminobenzoic acid ethyl ester hydrobromide (10·0 g, 31·53 mmol) was dissolved in 30 ml of water, an excess of saturated solution of sodium bicarbonate was added, and the separated amino ester was extracted by ethyl acetate. The acetate solution was dried by sodium sulfate and evaporated at reduced pressure. The residue was dissolved in a mixture of 10 ml of ethyl acetate and 16 ml of dimethylformamide, and benzyloxycarbonylisoleucine (7·35 g, 31·53 mmol) and dicyclohexylcarbodiimide (6·50 g, 31·53 mmol) were added at 0°C. After standing 1 h at 0°C and 48 h at room temperature, dicyclohexylurea which had separated was filtered off by suction and the filtrates were evaporated at reduced pressure. The dry residue became solid (13·1 g, 86%) after trituration with water. After recrystallization from aqueous ethanol and drying, 9·1 g (60%) was obtained of a product melting at 210—213°C (slow melting above 190°C, crystallization at 200°C, melting at 210—213°C). $[\alpha]_D^{25}$ —70·7° (*c* 4·8, acetic acid). For $C_{26}H_{33}.N_3O_6$ (483·5) calculated: 64·58% C, 6·88% H, 8·69% N; found: 64·50% C, 6·87% H, 8·51% N.

Isoleucyl-alanyl-*p*-aminobenzoic Acid Ethyl Ester (*I*)

Benzyloxycarbonylisoleucyl-alanyl-*p*-aminobenzoic acid ethyl ester (3·14 g, 6·5 mmol) was decarbobenzoxylated by the same procedure as the benzyloxycarbonyl-dipeptide ester. The hydrobromide was dried, dissolved in 5 ml of water, the solution was filtered, and made alkaline by the addition of a solution of sodium bicarbonate. The amino ester which had separated was extracted by ethyl acetate, the solution was dried by sodium sulfate, and evaporated at reduced pressure. The residue was recrystallized from ethyl acetate–light petroleum. The yield of isoleucyl-alanyl-*p*-aminobenzoic acid ethyl ester was 1·70 g (74%), m.p. 161—162°C, $[\alpha]_D^{25}$ —69·2° (*c* 2, methanol). For $C_{18}H_{27}N_3O_4$ (349·4) calculated: 61·87% C, 7·79% H, 12·03% N; found: 61·85% C, 7·75% H, 11·86% N.

REFERENCES

1. Zaoral M., Sláma K.: Science *170*, 92 (1970).
2. Bowers W. S.: Science *164*, 323 (1969).
3. Bowers W. S., Fales H. M., Thompson M. J., Uebel E. C.: Science *154*, 1020 (1966).
4. Muschaweck R., Habicht H. in the book: *Arzneimittel* (G. Erhart, H. Ruschig, Eds), Vol. 1, p. 253. Verlag Chemie, Weinheim 1968.
5. Knobler Y., Bittner S., Virov D., Frankel M.: J. Chem. Soc. *1969* (C), 1821.

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